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2   **Interpretive summary.** Use of on-farm data to guide treatment and control of mastitis caused by

3   *Streptococcus uberis,* by Samson *et al.* To reduce the risk of antimicrobial resistance, judicious use of

4   antimicrobials is advocated. We show that routinely available DHI and treatment data can be used in

5   veterinary practice to predict cure of *S. uberis* mastitis. Probability of apparent cure is higher among

6   1st and 2nd parity animals compared to older cows, and in animals with short-duration elevated SCC

7   compared to those with repeated SCC elevation before occurrence of mastitis. This knowledge

8   enables farmers and veterinarians to tailor antimicrobial use for treatment of mastitis, and to put

9   increased emphasis on prevention of cases with poor prognosis.

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Use of on-farm data to guide treatment and control of mastitis caused by *Streptococcus uberis*

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33 **ABSTRACT**

34

35 Treatment of mastitis is the most common reason for use of antimicrobials in dairy cattle. The

36 responsible use of antimicrobials could be strengthened by knowledge of predictors for cure, which

37 would help to tailor treatment decisions. Ideally, to allow for widespread uptake, this would be

38 achieved using data that are routinely available. To assess whether this is feasible in practice,

39 farmers were invited to submit milk samples from mastitis cases to their veterinary practice for

40 bacteriological culture. Among 624 culture-positive samples, 251 were positive for *Streptococcus*

41 *uberis*. Using cow-level data, cases were classified as severe clinical mastitis (CM; "severe"), 1st non-

42 severe CM ("first"), repeated non-severe CM ("repeat"), or subclinical mastitis ("subclinical").

43 Additional data were collected at cow-level (somatic cell count (SCC), parity, lactation stage, milk

44 yield, fat and protein content, treatment) and at herd-level (housing, bedding, pre-milking teat

45 disinfection, post-milking teat disinfection). Severe cases were overrepresented among heifers and

46 animals in early lactation whereas repeat cases were overrepresented in cows with 3 or more

47 lactations. The probability of cure was higher among 1st and 2nd parity animals than among older

48 cows, and higher in animals with a single elevated cow-level SCC than in animals with multiple high

49 SCC records. Results obtained in the current study are similar to those previously described for

50 *Staphylococcus aureus* mastitis. Thus, routinely available cow-level information can help to predict

51 the outcome of antimicrobial treatment of the most common causes of Gram-positive mastitis.

52

53 **Key words**: mastitis, *Streptococcus uberis*, prognosis, antimicrobial treatment, clinical manifestation

54 **INTRODUCTION**

55

56 Antimicrobial resistance is increasingly perceived as a threat to human and animal health and the

57 need for responsible use of antimicrobials is emphasized by a range of national and international

58 bodies (UK Department of Health 2013; World Health Organisation 2015). Key elements of the

59 approach proposed by the World Health Organisation (WHO) include reduction of the incidence of

60 infection and optimized use of antimicrobial medicines (WHO, 2015). Farmers are increasingly aware

61 of the need to use antimicrobials responsibly. In a recent survey in the UK over 70% of dairy farmers

62 said that reducing antibiotic usage would be "a good thing to do" (Jones *et al.*, 2015). Veterinarians

63 can play an important role in this process by providing information on ways to achieve reductions in

64 antibiotic usage, e.g. by minimizing the risk of disease or through development of treatment

65 protocols (Raymond *et al.*, 2006; Jones *et al.*, 2015).

66 On dairy farms, treatment of mastitis is a major reason for use of antimicrobials. For

67 example, Pol and Ruegg (2007) calculated the estimated overall exposure to antimicrobial drugs of

68 cattle on conventional dairy farms as 5.43 defined daily doses (DDD) per cow per year. This included

69 3.58 DDD of intramammary applications (2.02 DDD during lactation and 1. 56 DDD at dry off) and

70 1.85 DDD of parenteral use. Clinical mastitis (CM) was the most common reason for intramammary

71 or parenteral antimicrobial usage. To reduce the use of antimicrobials protocols for selective

72 treatment of dry cows and cattle with CM have been developed, including protocols based on

73 culture and on cow-factors such as SCC (Lago *et al.*, 2011; Cameron *et al.*, 2014; Scherpenzeel *et al.,*

74 2014). In culture-based protocols, treatment decisions are largely based on the distinction between

75 gram-positive growth, gram-negative growth and no growth (Lago *et al.*, 2011; Cameron *et al.*,

76 2014). Further refinement of treatment decisions may be possible when pathogen factors, such as

77 antimicrobial resistance, and host characteristics, including duration of infection and parity, are

78 taken into account, but this has only been described in detail for *Staphylococcus aureus* (Barkema *et*

79 *al.*, 2006).

80   In many countries on different continents *Streptococcus uberis* is among the most common

81   gram-positive causes of CM (Olde Riekerink *et al.*, 2008; Petrovski *et al.*, 2011; Verbeke *et al.*, 2014).

82   The organism is also responsible for a considerable proportion of subclinical mastitis cases (Bradley

83   *et al.*, 2007; Sampimon *et al.*, 2009). Intramammary infections and CM caused by *S. uberis* can be

84   transient, recurrent or chronic and a wide range of cure rates has been reported in response to

85   treatment (Zadoks *et al.*, 2003; Zadoks 2007). Despite its importance as a mastitis pathogen little is

86   known about risk factors for the clinical manifestation or treatment outcome of *S. uberis* IMI.

87   Therefore, the aim of this study was to generate data that could inform guidelines for improved

88   management of *S. uberis* mastitis under field conditions. To that end, we conducted a farm-based

89   study of herd- and cow-level risk factors that are associated with the clinical manifestation and

90   likelihood of apparent cure of *S. uberis* IMI as based on SCC. In doing so, we only used tools and data

91   that are routinely available to farmers and veterinary practices, including treatment and DHI records,

92   because our aim was to generate low-cost guidelines for improved management of *S. uberis* mastitis

93   under field conditions.

94 **MATERIALS AND METHODS**

95

96 *Milk sampling and Bacteriological culture*

97 From August 2012 until January 2014, quarter milk samples (n=624) were collected from French

98 dairy cows with clinical or subclinical mastitis using standard aseptic sample collection methods

99 (National Mastitis Council, 1999). Detection and sampling of mastitis cases was driven by

100 participating farmers. To motivate farmers to participate in the study, all clients of our veterinary

101 practice (Vetformance, Villaines la Juhel, France) with more than 50 lactating cows (approximately

102 500 farms), received an invitation to sample clinical and subclinical mastitis cases at their farms. The

103 bacteriological analysis of the samples was free of charge for the farmers. In addition, upon return of

104 completed data information sheets, a head collar for a cow was offered to the farmers. One hundred

105 and forty two farmers submitted at least one milk sample, indicating an uptake of approximately

106 28%.

107 Milk samples were subjected to bacteriological culture in the laboratory of Vetformance.

108 Aliquots of milk (10 µl) were plated onto three media, i.e. (1) Colombia blood agar containing 5%

109 sheep blood (bioMérieux, Craponne, France; Ref. 43041); (2) Colistin Nalidixic Acid (CNA) agar (blood

110 agar plate containing 5% sheep blood, Colistin (10 mg/L) and Nalidixic Acid (15 mg/L) (bioMérieux;

111 Ref. 43071) and (3) Bromo Cresol Purple (BCP) agar (bioMérieux; Ref. 43021). Plates were incubated

112 at 37°C for 18 to 24 h. Cultures were considered pure if only one morphotype was present on the

113 blood agar plate. For pure cultures, growth on both the CNA and BCP plates was considered as

114 evidence of the bacteria being gram-positive; growth on only the BCP plate as evidence of the

115 bacteria being gram-negative. Among gram-negative bacteria, *E.coli* was characterized by a positive

116 lactose reaction (colour change of the BCP plate from purple to yellow) and a negative urea reaction

117 (bioMérieux; Ref. 55752), whereas *Klebsiella* was identified by both positive lactose and positive

118 urea reactions. Gram-positive bacteria were considered to be *Staphylococcus aureus* based on

119 positive catalase and coagulase reactions (bioMérieux; Ref. 73112) and Staphylococcus spp. in the

120 case of positive catalase and negative coagulase results. Identification of *S. uberis* was based on

121 negative response in the catalase reaction and positive response in the esculin reaction (bioMerieux;

122 Ref 42086) (National Mastitis Council, 1999). In addition, susceptibility to penicillin was evaluated.

123 This procedure increases specificity by excluding enterococcal isolates, which are more likely to be

124 penicillin-resistant than streptococci (Makovec and Ruegg, 2003; Nam *et al.*, 2010). Susceptibility to

125 penicillin was tested using the disc diffusion method in accordance with the recommendations of the

126 Société Française de Microbiologie (Soussy, 2013). Using a swab, a Mueller Hinton agar plate

127 containing 5% sheep blood (MH2, bioMérieux; Ref. 43321) was homogenously plated with a

128 suspension of *S. uberis* at 0.5 McFarland (equivalent to approximately 10^8 colony forming units/ml).

129 Plates were incubated at 37°C for 18 to 24 h. Bacteria were considered sensitive to penicillin if they

130 expressed a growth inhibition zone of more than 21 mm around an Oxacillin disc (5 µg; Soussy,

131 2013). Bacteria that could not be classified using the criteria described here were considered "other

132 species".

133

134 ***Cow and Herd Data***

135 Three data sources were used to obtain information about individual cows and their herds of origin,

136 i.e. (i) private farm records on treatment; (ii) monthly DHI data; and (iii) questionnaires that were

137 filled out by the farmer and the attending veterinarian. For each cow the date of mastitis diagnosis

138 (observation of clinical mastitis or notification of SCC data via DHI) and treatment were recorded,

139 including use of intramammary administration of antimicrobials (IMM), parenteral administration of

140 antimicrobials (PAR), and use of non-steroidal anti-inflammatory drugs (NSAIDs). DHI data were

141 collected from the 3 milk recordings preceding the diagnosis of mastitis, the month of diagnosis and

142 the 3 recordings after diagnosis, if available. This included cow-level SCC data, parity (1 = first

143 lactation, 2 = second lactation, 3 = third or higher lactation), DIM, milk yield (MY, in kg), fat content

144 (g/kg), and protein content (g/kg). At herd level, information was collected on the use of pre-milking

145 teat disinfection (PreMTD) and post-milking teat disinfection (PostMTD), use of housing (yes or no)

146 and, where applicable, on housing type (cubicles or straw yards).

147

148 ***Classification of Cases***

149 Clinical manifestation was classified into four categories based on clinical severity of the current

150 episode and information on previous episodes of CM in the same animal:

151

152   1.   Severe: CM with both local and general symptoms (T > 39°C; temperature measured on

153   clinical suspicion of fever by the farmers and results recorded on the data form

154   accompanying the milk sample);

155   2.   Non-severe first case ("first"): first episode of CM during the current lactation with local

156   signs only (abnormalities of milk with or without abnormalities of the udder);

157   3.   Non-severe repeat case ("repeat"): 2nd or subsequent episode of CM during the current

158   lactation with local signs only in the current episode (abnormalities of milk with or

159   without abnormalities of the udder);

160   4.   Subclinical: SCC > 200,000 cells/ml at cow level based on most recent DHI data, not

161   accompanied by any clinical signs

162

163 For 212 of the 251 *S. uberis* positive animals SCC data were partially available (fewer than 3 records

164 before or after diagnosis) or complete (3 records before and after diagnosis). SCC data were used to

165 classify the duration of inflammation prior to diagnosis and the response to treatment after

166 diagnosis. Inflammation was considered short if at least 2 monthly SCC records prior to diagnosis

167 were below 200,000 cells/ml (SHORT) and long if at least 2 monthly SCC records exceeded 200,000

168 cells/ml prior to diagnosis (LONG). An animal was considered cured if at least 2 monthly SCC after

169 diagnosis were below 200,000 cells/ml (CURE) and not cured if at least 2 monthly SCC exceeded

170 200,000 cells/ml after diagnosis (NO CURE). For other SCC combinations or missing data, duration

171   and cure were not determined (ND), e.g. for animals in early lactation or for cows that were dried-

172   off or culled prior to completion of follow-up.

173

174   ***Statistical Analysis***

175   Statistical analyses were performed using Statistix, version 10 (Analytical Software, Tallahassee, FL).

176   Data were inspected for outliers and missing values and descriptive analyses were conducted using

177   tabular and graphical formats. For outcomes of interest with 3 or more categories, data were

178   analysed using categorical methods (Chi-Square analyses), e.g. for cow-level factors associated with

179   clinical severity. The association between clinical severity and milk, fat or protein yield relative to

180   occurrence of mastitis was evaluated using a t-test at each time point. To identify cow- and herd

181   level risk factors for apparent cure as based on SCC, logistic regression was used with backward

182   stepwise analysis. The final logistic regression equation was:

183

184   Logit (SCC cure) = intercept + Clinical manifestation + Duration + Parity + Treatment + error

185

186   where Clinical manifestation is severe, first, repeat or subclinical as defined above, Duration is the

187   inflammation history based on SCC (short, long, ND), parity is parity group (1, 2, 3+), DIM is

188   categorized into early, mid and late lactation (<100, 100-200, 200+) and treatment is treatment for

189   mastitis (IMM, PAR, and NSAIDs, or no treatment). Two way interactions between the main variables

190   were also evaluated for statistical significance. No correction was made for clustering of cases within

191   herd, because the model would not converge when herd was included due to the large number of

192   herds and the limited number of cases per herd. Goodness of fit of the final model was evaluated

193   using the model deviance and the Hosmer-Lemeshow statistic. In the Hosmer-Lemeshow statistic,

194   the data are divided into 10 approximately equal deciles of observed risk. In these deciles the

195   observed and expected number of observation are compared using a Chi-square distribution with

196   10-2 = 8 degrees of freedom (Hosmer and Lemeshow, 2013). A low value of the Hosmer-Lemeshow

197   statistic indicates a good fit to the data. In addition, a deviance value that is close to the remaining

198   degrees of freedom implies that there is **no evidence of a poor fit of the model to the data.**

199

200 **RESULTS**

201

202 ***Descriptive Analysis***

203 Of 624 milk samples submitted for culture 251 (40%) were positive for *S. uberis* in pure culture whilst

204 42 samples (7%) were culture negative. The remaining samples tested positive for *Escherichia coli* (n

205 = 108; 17%), *Klebsiella* (n = 12; 2 %)*, Staphylococcus aureus* (n = 76; 12%), *Staphylococcus* spp. (n =

206 103; 17%) or other species (n = 32; 5%). Samples positive for *S. uberis* originated from 142 farms.

207 From 99 farms, a single *S. uberis* positive sample was obtained whilst 20 and 23 farms provided 2 or

208 more *S. uberis* positive samples, respectively. All isolates originated from cows with clinical or

209 subclinical mastitis in one quarter with the exception of three cows where *S. uberis* was isolated

210 from two quarters on the same sampling date. The clinical manifestation of *S. uberis* positive

211 mastitis cases was significantly different from the clinical manifestation of *S. uberis* negative cases

212 (Chi-square = 38.0, df = 3, *P* < 0.005; Figure 1), with *S. uberis* overrepresented among non-severe

213 first cases and underrepresented among subclinical cases. Distribution across parities was not

214 different between *S. uberis* and other diagnoses (Chi-square = 1.56, df = 2, *P* = 0.46). During the first

215 100 days of lactation *S. uberis* was less common than other diagnoses, whereas it was more common

216 between 100 and 200 DIM (Chi-square=10.13, df = 2, *P* < 0.05). Milk yield, fat and protein content

217 were not different between *S. uberis* and non *S. uberis* cases prior to infection (results not shown).

218 Cow level data for *S. uberis* cases are summarized in Table 1. Severe and subclinical *S. uberis*

219 cases were overrepresented in parity 1 compared to higher parities, whereas non-severe first cases

220 were overrepresented in parity 2 and repeat cases in higher parities, respectively (Chi-square =

221 13.67, df = 6, *P* < .05). Severe cases were overrepresented in early lactation, whereas repeat cases

222 were overrepresented in mid-lactation (Chi-square = 13,02, df = 6, *P* < 0.05; Table 1). Treatment

223 records were available for ca. 80% of severe, first and repeat cases and for 42% of subclinical cases

224 (Chi-square = 22, df = 3, *P* = 0.0001). When no treatment was recorded, this was considered to

225   indicate that no treatment was administered. Intramammary antibiotics as the only treatment were

226   more commonly used to treat non-severe first cases as compared to severe, repeated and

227   subclinicial cases, whereas they were more commonly used in combination with parenteral

228   treatment for repeat cases and subclinical cases (Chi-square = 31; df =6, *P* < 0.0001; Table 1). The

229   combination of intramammary and parenteral antimicrobials with anti-inflammatory treatment was

230   mostly used in severe cases and never for subclinical cases (Table 1). Milk production was

231   numerically lower in severe cases than in non-severe cases, both before and after diagnosis of

232   clinical or subclinical mastitis, with the exception of yield at 3 DHI recordings prior to diagnosis, but

233   the difference was not significant. No differences were detected between severity classes with

234   regard to fat and protein content of milk before or after diagnosis of mastitis (data not shown).

235   Herd-level data was collected on farms with *S. uberis* positive results and is presented in

236   Table 2. Most herds were housed, either full time or part time. Straw yards were the predominant

237   housing system, with only 22% of herds housed in cubicles. PostMTD was used in almost all herds

238   and in more than half of all herds both PreMTD and PostMTD were used. Use of PreMTD without

239   PostMTD was not reported. Severe cases were overrepresented in herds without PostMTD (Chi-

240   square = 10.23, df = 3, *P* < .05).

241

242   ***Factors Associated with Cure of S. uberis IMI***

243   Cure was evaluated based on post diagnosis SCC values and results from the regression model are

244   shown in Table 3. A total of 125 cases had complete data and were included in this analysis. Model

245   deviance was 127.4 on 115 degrees of freedom, i.e. the values were similar and there was no

246   indication of a poor fit of the model to the data. In addition, the Hosmer-Lemeshow statistic was low

247   (6.05), implying a good fit to the data. The probability of cure was significantly higher in animals in

248   lactation 1 and 2 compared to older animals. The probability of cure increased numerically with

249   increasing number of treatment types, i.e. from no treatment to intramammary antimicrobials only

250   to combined intramammary and parenteral antimicrobials, to both routes of antimicrobial

251   administration combined with NSAID. However, there was no statistically significant difference in

252   cure between treatments. Finally, the probability of cure was higher among IMI with a short history

253   of inflammation than those with a long history of inflammation prior to treatment (Table 3). Clinical

254   manifestation and herd level variables were not associated with cure.

255

256 **DISCUSSION**

257 In this study we aimed to use routinely available herd and animal-level data to support control of *S.*

258 *uberis* mastitis and the judicious use of antimicrobials. Risk factors for the incidence of *S. uberis*

259 mastitis (clinical mastitis or IMI) have been described at herd-level (Barkema *et al.*, 1999; Ericsson

260 Unnerstad *et al.*, 2009) and animal-level (Zadoks *et al.*, 2001; Breen *et al.*, 2009), and the impact of

261 different treatment regimens on the outcome of treatment of *S. uberis* mastitis has been described

262 for experimentally induced (Hillerton and Kliem, 2002; Oliver *et al.*, 2003) and naturally occurring

263 infections (reviewed in Zadoks, 2007). To our knowledge, animal-level risk factors for severity of

264 disease or treatment outcome of *S. uberis* IMI have not been described. Here, we show for the first

265 time that animal-level data can be used to predict the outcome of antimicrobial treatment of *S.*

266 *uberis* mastitis and to guide treatment decisions. Specifically, the probability of cure was higher

267 among 1st and 2nd parity animals compared to older cows, and in animals with at most a single

268 elevated cow-level SCC before diagnosis compared to those with multiple high SCC records. Those

269 findings are strikingly similar to results obtained for *Staphylococcus aureus* IMI across a range of

270 studies covering both clinical and subclinical mastitis (reviewed in Barkema *et al.*, 2006) and can be

271 used to inform decisions about treatment duration or the choice between treatment and culling. The

272 individual making treatment decisions will be able to weigh these factors in the decision making and

273 to use this information to provide a realistic prognosis. Pathogen-specific predictors for cure, as

274 described here for *S. uberis*, are particularly useful when information on the causative agent is

275 available. Several studies have demonstrated the feasibility of using on-farm diagnostics to inform

276 case management (Lago *et al*., 2011; Cameron *et al*., 2014) and additional tests for rapid or on-farm

277 screening of milk samples are under development, including culture and DNA-based tests (Viora *et*

278 *al*., 2014; Bosward *et al*., 2016). Considering the similarities between results obtained for *S. uberis*

279 and *S. aureus*, some of this information may also be of value in the absence of an etiological

280 diagnosis, although further field evaluation will be needed to validate such a generic approach.

281   Increased parity was associated with a reduced likelihood of cure. This is not merely a

282   reflection of the chronicity of infection, because parity was significant after correction for SCC, which

283   is a proxy for duration (Barkema et al., 2006). The mechanism behind reduced probability of cure in

284   older animals is unknown. Possible explanations were discussed by Barkema and co-workers (2006)

285   for the response to treatment of *Staph. aureus* IMI. One potential explanation is the change in ratio

286   between udder volume, which increases with age, and the administered dose of antimicrobials,

287   which is independent of age, resulting in a lower dose per unit udder volume in older animals

288   (Barkema *et al.*, 2006). This reasoning would also apply to *S. uberis* treatment. Immunosenescence,

289   the waning of the immune response with age, could be postulated to play a role in deterioration of

290   treatment outcome with age, but there is no specific evidence for this in the context of bovine

291   mastitis. Regardless of the underlying mechanism, the comparatively poor treatment response of

292   older cows can be interpreted as an imperative to help our cows to age healthily, e.g. by selecting

293   for cows with high genetic merit for udder health or immune responsiveness (Thompson-Crispi *et al.*,

294   2014). In addition, animal-level risk factors should be minimized where possible. For example,

295   severe teat end hyperkeratosis is an animal-level risk factor for *S. uberis* CM, and the risk of

296   hyperkeratosis can be reduced by avoiding overmilking (Breen *et al.*, 2009; Edwards *et al.*, 2013).

297   The observation that duration of IMI, as measured by number of elevated monthly SCC prior

298   to treatment, is predictive of cure is compatible with previous data on both *S. uberis* and *Staph.*

299   *aureus*. A detailed longitudinal study of *S. uberis* IMI in 2 herds showed that some episodes of CM

300   are due to recent IMI, whereas other CM episodes are preceded by periods of elevated (Zadoks *et*

301   *al.,* 2003). CM episodes without preceding SCC elevation were more likely to be followed by cure

302   than CM episodes with preceding SCC elevation. Similarly, in several treatment trials of *Staph.*

303   *aureus* IMI, higher or longer SCC elevation prior to treatment was associated with a decreased

304   probability of cure (reviewed in Barkema *et al.*, 2006). A poor response of chronic *Staph. aureus* IMI

305   to treatment may be explained in part by micro-abscess formation and fibrosis (Erskine *et al.*, 2003).

306   Fibrosis also occurs during *S. uberis* mastitis, starting as early as 6 days after infection in

307   experimental challenge studies. It is accompanied by presence of the pathogen in subepithelial and

308   septal tissue and lymphatic vessels and lymph nodes (Thomas *et al.*, 1994). This may explain why the

309   response of *S. uberis* mastitis to treatment can be poor, even after extended therapy (Milne *et al.*,

310   2005). Both in experimentally induced and in persistent *S. uberis* IMI, extended therapy increases

311   the probability of cure (Oliver *et al.*, 2003; Swinkels *et al.*, 2014). The benefits of extended therapy

312   must be weighed against its disadvantages, including increased costs of antibiotics and milk discard,

313   and increased risk of residue in milk and selection for antimicrobial resistance (Hillerton and Kliem,

314   2002; Barkema *et al.*, 2006). As in any risk factor study, the risk factors identified in the current

315   study, including treatment modality, and their coefficients allow us to quantify the increase or

316   decrease in the likelihood of a particular treatment outcome, but the specific outcome in any

317   individual animal cannot be predicted.

318   In the current study a numerical but non-significant increase in cure was observed with an

319   increase in treatment modalities (intramammary and parenteral antimicrobials and NSAIDs). This

320   study was not, however, a randomized controlled clinical trial, nor was it meant to be. Farmers

321   tended to treat severe cases of mastitis with a combination of intramammary, parenteral and anti-

322   inflammatory products, first clinical cases with intra-mammary treatment only and repeated- and

323   subclinical cases with antimicrobial treatment by both the intra-mammary and parenteral route.

324   This implies farmers' awareness of the usefulness of cow-specific treatment, with consideration of

325   both animal welfare and economic aspects of treatment. This information provides evidence that

326   farmers are willing to make cow-specific decisions and bodes well for the feasibility of including cow-

327   specific risk factors in future protocols. In our practice, farm specific treatment protocols are already

328   discussed with each farmer on an annual basis and the treatment choices reported by the farmers

329   are in line with those protocols. As a next step towards judicious use of antimicrobials, we envisage

330   implementation of cow-specific protocols.

331   Animals with short duration mastitis (no or single SCC elevation prior to diagnosis) were

332   likely to cure (no or single SCC elevation after diagnosis), whereas animals with long duration

333   mastitis (multiple SCC elevation prior to diagnosis) were likely not to cure (multiple SCC elevations

334   after diagnosis). Similarly, data availability post-diagnosis mirrored data availability pre-diagnosis, i.e.

335   animals with incomplete SCC data prior to diagnosis often had incomplete SCC data after diagnosis

336   too (data not shown). This would mostly apply to animals in early lactation that were lost to follow-

337   up due to culling. Thus, although our analysis shows no significant difference in cure between

338   different severity classes, this result is affected by "healthy worker bias", whereby only surviving

339   cows are included in the analysis. Indeed, loss to follow-up as indicate by absence of data on cure

340   was proportionally higher for severe cases than for non-severe cases (Table 1).

341   Of the herd-level factors considered in this study, use of PostMTD was associated with a

342   reduced risk of severe, repeat and subclinical *S. uberis* mastitis compared to first cases of mastitis.

343   The value of PostMTD in reducing the risk of *S. uberis* IMI has been documented repeatedly (Zadoks

344   et al., 2003; Galton, 2004; Williamson and Lacy-Hulbert, 2013) but it has not been linked to clinical

345   manifestation. Strain-specific transmission and virulence patterns have previously been suggested or

346   documented (Zadoks et al., 2003; Tassi et al., 2013) and could theoretically contribute to an

347   association between PostMTD and clinical manifestation. It has also been hypothesized that host

348   immune status may contribute to clinical manifestation of *S. uberis* IMI (Tassi et al., 2013). Indeed, in

349   the current study, severe cases of CM were overrepresented among heifers and animals in early

350   lactation. This emphasizes the importance of another herd-level management factor, i.e. adequate

351   care for non-lactating animals. Considering that *S. uberis* is common in the faeces and environment

352   of cattle (Zadoks *et al.*, 2005), environmental hygiene is of particular importance. The risk of

353   infection in heifers and dry cows can also be reduced through use of teat spray and internal teat

354   sealants, respectively (Lopez-Benavides *et al.*, 2009; Compton *et al.*, 2014). With increasing pressure

355   to reduce antimicrobial use, implementation of non-antimicrobial mastitis prevention measures

356   becomes increasingly important.

357   In this field study, definitions of transient and persistent IMI and cure were based on SCC

358   data. Although repeated post-treatment culture has been considered the "gold standard" for cure in

359   clinical trials, additional or alternative metrics for cure are increasingly reported in field studies. SCC

360   has been used as a primary criterion for cure in studies of clinical mastitis, subclinical mastitis, and

361   dry cow treatment (St. Rose *et al.*, 2003; Lago *et al.*, 2011; Persson *et al.*, 2015). SCC is routinely used

362   as an indicator of infection status (Schukken *et al.*, 2003), although the probability of bacteriological

363   cure is higher than the probability of SCC-based cure in studies of chronic streptococcal mastitis (St.

364   Rose *et al.,* 2003). SCC is of immediate interest to farmers, unlike bacteriological cure which is

365   primarily of academic interest. Moreover, SCC is routinely available at very low cost, which makes its

366   large-scale use feasible in field studies, veterinary practice and farm management. Finally, SCC

367   captures long-term outcomes of mastitis treatment, whereas culture results generally only reflect

368   the first few weeks post-treatment. Thus, SCC is a convenient, affordable and meaningful indicator

369   of treatment outcome.

370   In conclusion, we show that treatment recommendations can be informed by animal-level

371   data that is routinely available to farmers and veterinarians, such as parity and SCC. To some extent,

372   treatment recommendations can be animal-specific rather than pathogen-specific, as both *S. uberis*

373   IMI and *Staph. aureus* IMI show a better response to treatment in animals in first or second lactation

374   and in animals with a single high SCC than in older animals or animals with multiple high SCC values

375   prior to treatment. In older animals or animals with multiple high SCC values the simultaneous use of

376   multiple treatment modalities may enhance the probability of cure but this would result in increased

377   use of antimicrobials. To limit the need for such treatment, continued or renewed emphasis on herd

378   management and infection prevention is needed. Formal validation of the observations described

379   here through a randomized controlled clinical trial may strengthen the evidence base underpinning

380   the suggested treatment decisions. In the absence of such validation, the evidence presented here is

381   the best available evidence to inform decisions on treatment of *S. uberis* mastitis, the most common

382   type of mastitis observed in this and many other studies.

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496

497   **Figure legend**

498   **Figure 1.** Clinical manifestation of mastitis for quarters with *S. uberis* negative (n = 373, white) and *S.*

499   *uberis* positive (n =251, black) milk samples (Chi-square = 38.0, df = 3, *P* < 0.005). Severe: clinical

500   mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical suspicion of fever);

501   Non-severe first case: first occurrence of CM during the current lactation with local signs only

502   (abnormalities of milk with or without abnormalities of the udder). Non-severe repeat case: repeat

503   occurrence of CM during the current lactation with local signs only during the current episode;

504   Subclinical mastitis: elevated cow-level SCC (> 200.000 cells/ml based on DHI data) not accompanied

505   by any visible abnormalities.

506

Classification of mastitis based on clinical expression
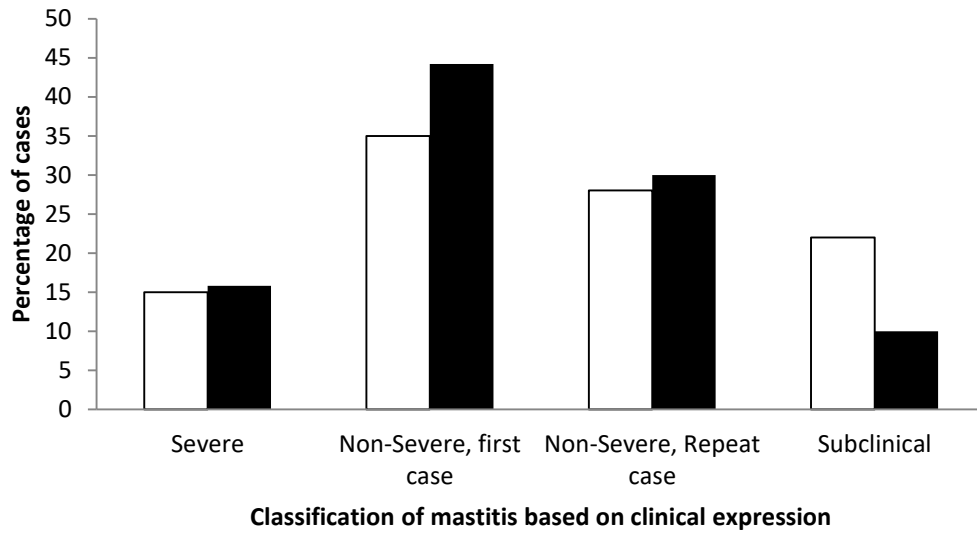
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Samson *et al.*, Figure 1.

523 **Table 1.** Cow- level data for *S. uberis* positive mastitis cases (number and (%)) with break-down by

524 manifestation. Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked

525 upon clinical suspicion of fever); First: first occurrence of CM during the current lactation with local

526 signs only (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat

527 occurrence of CM during the current lactation with local signs only during current episode;

528 Subclinical: cow-level SCC (> 200,000 cells/ml based on DHI data) not accompanied by any signs.

529

Cow factor	All *S. uberis* cases, n (%)	*S. uberis* cases by clinical manifestation, n (%)			
		Severe	First	Repeat	Subclinical
DIM					
<100	123 (100)	26 (21)	56 (46)	27 (22)	14 (11)
100 to 200	63 (100)	7 (11)	25 (40)	27 (43)	4 (6)
> 200	50 (100)	4 (8)	24 (48)	17 (34)	5 (10)
subtotal	236 (100)	37 (16)	105 (44)	71 (30)	23 (10)
Parity					
First	61 (100)	12 (20)	23 (38)	16 (26)	10 (16)
Second	55 (100)	7 (13)	34 (62)	12 (22)	2 (4)
Higher	122 (100)	17 (14)	49 (40)	44 (36)	12 (10)
subtotal	238 (100)	36 (15)	106 (45)	72 (30)	24 (10)
Treatment[1]					
none	49 (100)	7 (14)	17 (35)	12 (24)	13 (27)
IMM	67 (100)	6 (9)	42 (63)	17 (25)	2 (3)
IMM + PAR	103 (100)	14 (14)	41 (40)	40 (39)	8 (12)
IMM + PAR + NSAID	19 (100)	10 (53)	6 (32)	3 (16)	0 (0)
subtotal	238 (100)	37 (16)	106 (45)	72 (30)	23 (10)

Duration[2]					
Short	78 (100)	8 (10)	35 (45)	30 (38)	5 (6)
Long	66 (100)	8 (12)	25 (38)	23 (35)	10 (15)
ND	96 (100)	22 (23)	46 (48)	19 (20)	9 (9)
subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)
Cure					
Yes	79 (100)	10 (13)	39 (49)	23 (29)	7 (9)
No	97 (100)	12 (12)	38 (39)	33 (34)	14 (14)
ND	64 (100)	16 (25)	29 (45)	16 (25)	3 (5)
subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)

530

531   [1.] Treatment: IMM = intra-mammary antibiotic treatment, PAR= parenteral antibiotic treatment,

532   NSAID = non-steroidal anti-inflammatory drugs.

533   [2.] Duration: Short = at least 2 monthly cow-level SCC < 200,000 cells/ml before diagnosis; Long =

534   at least 2 monthly cow-level SCC > 200,000 cells/ml before diagnosis; ND = not determined due

535   to insufficient SCC data before diagnosis.

536   [3.] Cure: Yes = at least 2 monthly cow-level SCC < 200,000 cells/ml after diagnosis; Long = at least 2

537   monthly cow-level SCC > 200,000 cells/ml after diagnosis; ND = not determined due to

538   insufficient SCC data after diagnosis.

539   **Table 2**. Herd-level data for *S. uberis* positive mastitis cases (number and (%)) with breakdown by

540   manifestation. Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked

541   upon clinical suspicion of fever); First: first occurrence of CM during the current lactation with local

542   signs only (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat

543   occurrence of CM during the current lactation with local signs only during current episode;

544   Subclinical: cow-level SCC (> 200.000 cells/ml based on DHI data) not accompanied by any signs.

545

Herd factor	All *S. uberis* cases,	*S. uberis* cases by clinical manifestation, n (%)			
	n (%)	Severe	First	Repeat	Subclinical
Housing					
Permanent	120 (100)	15 (13)	57 (48)	39 (33)	9 (8)
Partial	92 (100)	20 (22)	32 (35)	28 (30)	12 (13)
None	28 (100)	3 (29)	17 (61)	5 (18)	3 (11)
subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (10)
Bedding					
Straw yard	187 (100)	27 (11)	84 (45)	58 (31)	18 (75)
Cubicles	53 (100)	11 (21)	22 (42)	14 (26)	6 (25)
Subtotal	240 (100)	38 (16)	106 (44)	72 (30)	24 (100)
Pre-dipping					
Yes	130 (100)	19 (15)	56 (43)	44 (34)	11 (8
No	106 (100)	18 (17)	48 (45	27 (25)	13 (12)
Subtotal	236 (100)	37 (16)	104 (44)	71 (30)	24 (10)
Post-dipping					
Yes	214 (100)	29 (14)	94 (44)	67 (31)	24 (11)
No	22 (100)	8 (36)	10 (45)	4 (18)	0 (0)
Subtotal	236 (100)	37 (16)	104 (44)	71 (30)	24 (10)

546

547 **Table 3**. Logistic regression of cow-factors versus cure for 125 cases of *S. uberis* mastitis.

548

Variable	Coefficient (SE)	Odds Ratio	95% C.I. for Odds Ratio	Z-value	P-value
Constant	-4.8 (1.3)			-3.68	0.0002
Parity					
First	1.7 (0.6)	5.5	1.8 to 5.5	3.1	0.0023
Second	1.3 (0.6)	3.8	1.3 to 11.1	2.42	0.016
Third or higher	Base				
Clinical manifestation[1]					
Severe	1.6 (1.3)	4.8	0.3 to 68.7	1.16	0.24
First	2.0 (1.2)	7.1	0.7 to 77.0	1.65	0.10
Repeat	1.9 (1.2)	6.4	0.6 to 70.9	1.54	0.13
Subclinical	Base				
Duration [2]					
Short	1.7 (0.5)	3.1	2.2 to 14.2	3.74	0.0002
Long	Base				
Treatment[3]					
IMM + PAR + NSAID	2.2 (1.3)	9.4	0.7 to 9.4	1.68	0.09
IMM + PAR	1.1 (0.6)	3.1	0.9 to 3.1	1.79	0.074
IMM	0.7(0.7)	2.1	0.6 to 8.2	1.09	.28
None	Base				

549

550   [1.]   Severe: clinical mastitis (CM) with local and general symptoms (T > 39°C; checked upon clinical

551   suspicion of fever); First: first occurrence of CM during the current lactation with local signs only

552   (abnormalities of milk with or without abnormalities of the udder). Repeat: repeat occurrence

553   of CM during the current lactation with local signs only during current episode; Subclinical: cow-

554   level SCC (> 200.000 cells/ml based on DHI data) not accompanied by any signs.

555   2. Duration: Short = at least 2 monthly cow-level SCC < 200,000 cells/ml before diagnosis; Long =

556   at least 2 monthly cow-level SCC > 200,000 cells/ml before diagnosis; ND = not determined due

557   to insufficient SCC data before diagnosis.

558   3. Treatment: IMM = intra-mammary antibiotic treatment, PAR= parenteral antibiotic treatment,

559   NSAID = non-steroidal anti-inflammatory drugs.